REMARKS

Claim 24 has been amended and new Claims 62-80 have been added to point out with more particularity and clarity the subject matter regarded by the Applicant as his invention. Applicant respectfully submits that no new matter has been entered by the above amendments and new claims.

Independent Claim 24, from which Claims 25-72 depend, has been amended in one aspect to clarify that the disease or disease susceptibility trait is known to be associated with the two or more subject genes, and that if there is an about 50% decrease in the level of wild-type protein normally expressed by a subject gene that that subject gene contains the germline mutation, and the organism is affected by the disease or disease susceptibility trait associated with the germline mutation in that gene. The amendments to claim 24 point out that each of the subject genes is associated with a disease or a disease susceptibility trait, in contrast to new independent Claim 73, from which Claims 74-80 depend, wherein the ratio is between the wild-type protein expressed by a subject gene that is suspected of containing a germline mutation known to be associated with a disease or a disease susceptibility trait, and a wild-type protein expressed by a reference gene that is not suspected of containing a germline mutation known to be associated with a disease or disease susceptibility trait.

One of the premises upon which the instantly claimed invention is based is that it would be unusual for an organism to contain more than one germline mutation in its genome. The Summary of the Invention at page 5, line 32 to page 6, line 2 states: "The immunoassay methods of this invention are also in this aspect premised on the assumption that germline mutations in two different genes of one individual are very rare." [Emphasis added.]

In another aspect, Claim 24 is amended for particularity and clarity to specify that the type of germline mutations that are known to be associated with diseases or disease susceptibility traits are "selected from the group consisting of truncation-causing mutations, mutations that cause allelic loss, and mutations that cause the expression of proteins with non-wild-type epitopes. . . ." [Claim 24 as amended.] Support for that amendment can be found at least at page 7, line 6 to page

8, line 7, wherein "[r]epresentative genes subject to truncation-causing mutations and/or allelic loss, and the disease(s) associated with mutations in such genes are listed. . . ." [Specification, page 7, line 10 to page 8, line 1]; at page 9, lines 22-25; at page 11, lines 24-25; at page 23, lines 3-4; at page 23, line 12 to page 24, line 20; at page 26, lines 24-31; at page 27, lines 10-16; at page 29, lines 22-23; at page 31, line 7 to page 36, line 29; at page 46, lines 9-11; and at page 47, lines 9-12.] The original Claim 14 (now canceled, but reflected in previously presented Claim 61) reads: "The method of Claim 1 wherein said mutation is or said mutations are selected from the group consisting of truncating-causing mutations and mutations that cause allelic loss."

Although the Specification states at page 23, lines 12-13: "A potential limitation of the assays of this invention are that they do not detect mutations, such as, missense mutations, that result in a full-length gene product . . . ," the Specification qualifies that statement at page 24, lines 3-20 which reads:

> Further, in **non-truncating missense mutations** that involve substitution of an amino acid, a change which should not alter the overall length of the polypeptide backbone of the protein, different approaches can be used to detect those mutations if immunoassay is used for diagnosis. **For example, an important potential consequence of an amino acid substitution (in regard to the ability to detect them) is that such a substitution may alter the three dimensional native protein structure to a sufficient extent that it changes the epitope exposure in native MMR proteins, and this may be detectable by immunoassay using antibodies to specific epitopes**.
>
> Thus, the flow cytometric immunoassay may be capable of detecting some types of protein alterations due to missense mutations since flow cytometry involves analysis of permeabilized cells, a situation in which the 3-D native MSH2 and MLH1 protein structures should be preserved. Such mutations could be detected by alternative strategies because assays for MMR mutations are being developed in other laboratories which are based on detecting functional changes in enzymatic activity for cellular mismatch repair [Bennett et al., <u>Cancer Res., 57</u>: 2956 (1997)]. Another possibility includes detecting changes in protein mobility using 2-D gel electrophoresis systems or high performance liquid chromatography (HPLC) analogous to assays for

> diagnosing hemoglobinopathies [ESA, Inc.; Chelmsford, MA (USA)].

[Emphasis added.]

New Claims 62-72 dependent on Claim 24 are directed to the method of Claim 24, wherein the two or more subject genes are associated with the same disease or disease susceptibility trait. Support for new Claim 62 can be found at least at page 8, lines 8-22, particularly at lines 14-22, which state:

> The reference protein can be . . . <u>the expression product of another subject gene that could also be associated with the disease or disease susceptibility to which the assay is directed</u>. In that latter embodiment, the assay could be characterized as a form of differential diagnosis/prognosis, determining in one assay which of several genes is affected by a disease-associated mutation.

[Emphasis added.] Further support for added Claim 62 can be found at page 10, lines 3-26.

Support for Claims 63-66, directed to specific subject genes associated with the same specific disease or disease susceptibility trait, can be found throughout the specification, and at the least at page 6, lines 4-20; at page 7, line 6 to page 8, line 1; and at page 38, line 21 to page 39, line 15, particularly at page 38, lines 21-25. Particular exemplary support can be found at the least at page 8, lines 20-22, which states: "Exemplary thereof is the assay method specifically described herein, wherein MLH1 and MLH2 are the subject genes, and susceptibility to HNPCC is the trait to which the assay is directed."

A distinct embodiment of the instant invention is now separately claimed in dependent Claims 67-72, wherein the two or more subject genes are associated with different diseases or disease susceptibility traits. Support for added Claim 67 can be found at least at page 5, line 32 to page 6, line 4, which states:

> The immunoassay methods of this invention are also in this aspect premised on the assumption that germline mutations in two different genes of one individual are very rare.
>
> Representative immunoassays of this invention are those to detect susceptibility to HNPCC and FAP in humans.

Additional support for Claim 67 can be found particularly at page 6, lines 29-31, which states: "In one aspect, the immunoassays of this invention are designed to identify carriers of hereditary traits associated with disease, such as the hereditary HNPCC and FAP traits"; and at page 7, lines 6-9, which states:

> The immunoassays of this invention may be adapted to measure full-length (wild-type) proteins associated with many other hereditary and genetic disorders (cancer and noncancer) that are due to mutations that cause protein truncation (germline and acquired) or cause the absence of allelic protein expression.

Support for Claims 69-72, directed to specific subject genes associated with specific diseases or disease susceptibility traits that are different, can be found throughout the specification, and at the least at page 6, lines 3-4, at page 7, line 6 to page 8, line 1, and at least in the listing at page 31, line 21 to page 37, line 2 of different exemplary "hereditary and genetic disorders (cancer and non-cancer) that are due to mutations that cause protein truncation . . . or cause the absence of allelic protein expression . . . ." [Specification, page 31, lines 8-10.]

New independent Claim 73 from which Claims 74-80 depend has been added for particularity and clarity to claim separately an embodiment that had previously been incorporated in independent Claim 24. The embodiment of new Claims 73-80 is that wherein the amount of wild-type protein of a subject gene that is associated with a disease or a disease susceptibility trait is compared to the amount of wild-type protein expressed by a reference gene that is <u>not</u> known to be associated with a disease or a disease susceptibility trait.

As indicated at page 8, lines 14-15: "the reference protein can be unrelated to the disease or disease susceptibility trait to which the assay is directed. . . ." Additional support for new Claims 73-80 can be found throughout the specification, for example, at least at page 38, lines 21-25 which states:

> Various modifications to said basic automated immunoassay format can be easily envisioned by ones of skill in the art. For example, such an assay format could be varied such that two gene products, e.g., MLH1 and MSH2, or a gene product and <u>reference protein, e.g., APC and tubulin, β-actin</u>

> or GAPH, could be detected and quantitated in the same cell lysate sample, preferably simultaneously.

[Emphasis added.] The specification specifically points out at page 9, lines 13-15, that "[a] preferred exemplary reference protein . . . is actin, tubulin, or glyceraldehyde-3-phosphate dehydrogenase."

Support for new Claims 76-78, directed to specific diseases and specific subject genes, wherein the amount of wild-type protein expressed by a subject gene associated with a disease or a disease susceptibility trait is related to the amount wild-type protein expressed by a reference gene not associated with a disease or disease susceptibility trait, can be found throughout the specification, and at the least at page 7, line 6 to page 8, line 1, and at page 31, line 6 to page 37, line 2. New Claims 76 and 77 reflect original Claims 38 and 39.

Applicant respectfully concludes that no new matter has been entered by the above amendments to Claim 24 and by the addition of new Claims 62-80.

35 USC 112, 1st Paragraph Rejection

Claims 24-28, 32-35, 37-44, 55-57 and 59-61 stand rejected under 35 USC 112, first paragraph because "[t]he claim(s) contains subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." [Office Action, page 3, section 6.] Applicant respectfully traverses this rejection, pointing out that independent Claim 24 has been amended to point out with more particularity and clarity the subject matter regarded by the Applicant as his invention, and that the only other pending independent claim, that is, new Claim 73, reflects those amendments.

Those amendments identify with more particularity and clarity the types of germline mutations that are detected by the methods of the invention namely, "truncation-causing mutations, mutations that cause allelic loss, and mutations that cause the expression of proteins with non-wild-type epitopes . . ." [Claims 24 and 73.] Applicant respectfully submits that the genus of diseases and disease susceptibility traits is thereby identified with more particularity and clarity.

The PTO has the initial burden of proof to present "evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims." [Synopsis of Application of Written Description Guidelines ("Synopsis"), page 4; http://www.uspto.gov/web/menu/written.pdf.] Applicant respectfully submits that the PTO has not met that burden.

The Office Action only proffers the data in Bouffler as "evidence" to meet that burden of proof. The Office Action states at page 4:

> Furthermore, there is evidence that the mutations of genes contemplated by applicant at the time of filing, and specifically claimed as being included within the scope of the broadest claim 24, would not fit the criteria of being known to be associated with a disease through the process of producing an about 50% decrease in level of wild-type protein. Msh2 is one of the subject genes listed in claim 38, that is dependent from claim 24, and should therefore, be a gene, the germline mutation of which causes an about 50% decrease in level of wild-type protein. However, Bouffler . . . teaches that Western blot analysis fails to detect a difference in Msh2 protein levels between wild type and heterozygote cells. . . .

Applicant respectfully points out that that characterization of Bouffler in the Office Action is in error. Bouffler describes a mouse model for HNPCC. The mutation in the mouse model is a targeted deletion of exon 7 in the mouse *Msh2* gene (and not the human MSH2 gene claimed in Claim 38). That model was used to investigate the role of *Msh2* in chromosome stability, partly to determine whether somatic mutation of the second wild-type allele occurred at a normal frequency, or at an increased frequency due to the mismatch repair defect. That study showed a phenotypic effect of haploinsufficiency of the mouse Msh2 gene (45% reduction of MNU-induced sister-chromatid exchanges in the $Msh2^{+/-}$ cells, compared to wild-type).

Applicant respectfully submits that Bouffler does not suggest that the artificially created Msh2 mutation in the mouse model accurately represents the types and proportions of MSH2 mutations in a human population. Furthermore, the Western blot analysis of Msh2, to which the Office Action refers, was taken somewhat out of context. Bouffler states at page 1292 (col. 2):

> Thus, *Msh2* shows haploinsufficiency in this assay. Western analysis does not detect a difference in *Msh2* protein level between wild type and heterozygote ES cells (RF, unpublished observation) most probably due to limitations of sensitivity.

[Emphasis added.] By that last underlined phrase, Bouffler implies that, given more favorable assay conditions, e.g., the presence of sufficient protein in the cell samples, the author would expect to see a difference in *Msh2* protein level "between wild type and heterozygote ES cells." Bouffler does not state how many mouse cells were used to prepare these Western blot samples; it is quite possible that there simply was not enough protein in the mouse cell samples to detect significant differences in protein levels between the wild type and heterozygote samples. The fact that Bouffler did not publish the data for the observation suggests that Bouffler did not consider the data conclusive.

Applicant respectfully concludes that Bouffler, for the reasons detailed above, does not provide

> evidence that the mutations of genes contemplated by applicant at the time of filing, and specifically claimed . . . would not fit the criteria of being known to be associated with a disease through the process of producing an about 50% decrease in level of wild-type protein.

[Office Action at page 4.] Applicant further respectfully concludes that the PTO has not met its burden of proof that "a person skilled in the art would not recognize that the written description of the invention provides support for the claims." [Synopsis, supra.]

Applicants respectfully further conclude that there is no reason to doubt the adequacy of the instant application's written description, but arguendo, even if the PTO had met its initial burden of proof, that Applicant could rebut that evidence with additional evidence, such as, the two references cited on PTO form 1449 accompanying this response. Those articles are

- Fields et al., J. Lab. Clin. Med., 143(1): 60 (Jan. 2004) ("Fields et al."); and
- Shigeta et al., Cancer Res., 59: 2602-2607 (June 1, 1999) ("Shigeta et al.").

Fields et al. shows that MLH1 and MSH2 do fit the criteria of the instant invention, wherein a number of different germline mutations are identified by the ratios

of MSH1/MSH2 or MSH2/MLH1 resulting in an about 50% decrease (within statistical error) in the wild-type protein expressed by the subject gene affected by a germline mutation. Table II at page 63, col. 2 exemplifies such representative results. Fields et al. then directly removes any residual doubt that Bouffler could have cast on the adequacy of the instant application's written description.

Shigeta et al. exemplifies the effectiveness of the embodiment of the invention claimed in independent Claim 73, wherein the amount of wild-type protein expressed by a subject gene is in ratio with the amount of wild-type protein of a reference gene, wherein the reference gene is not known to be associated with a disease or a disease susceptibility trait. Shigeta et al. found that normal lymphoblastoid cells derived from heterozygous carriers of ATM mutations produced, on average, about 50% less wild-type ATM protein than unaffected individuals; □-tubulin protein was used as an internal control for Western blot analysis.

Applicant respectfully points out that the Guidelines for the Examination of Patent Applications Under the 35 USC 112, ¶ 1, "Written Description" Requirement [hereinafter cited as "Guidelines"]; Fed. Register, 66(4) (January 5, 2001) at page 1105, column 1] notes under the sub-heading "A. Original Claims":

> There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed. . . . However, the issue of lack of adequate written description may arise even for an original claim when an aspect of the claimed invention has not been described with sufficient particularity such that one skilled in the art would recognize that the applicant had possession of the claimed invention. . . . The claimed invention as a whole may not be adequately described if the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art. . . .

[Emphasis added.] The Guidelines [*id.* at page 1106, column 1] indicate that "the description need only describe in detail that which is new or not conventional." [Emphasis added.]

Applicant respectfully points out that it is conventional in the art to prepare antibodies to a protein, that is, to any protein, whether newly discovered or not. [See,

for example, Ex Parte Erlich, 3 USPQ2d 1011 (PTO Bd. Pat. App. & Int'f. 1987); In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988); Hybritech, Inc. v. Monoclonal Antibodies, Inc., 231 USPQ 81 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987).] Further, it is conventional in the art to avoid detecting other than full-length wild-type proteins in an immunoassay. Suitable antibodies, if not commercially available, can be prepared and screened for any antigen [id.], and depending on the assay format to be used, suitable antibodies can be selected to detect only wild-type full-length proteins based on conventional knowledge in the art. For example, if the germline mutation is known to be a truncation-causing mutation, an antibody to the carboxyl end of the subject wild-type protein could be sufficient.

As indicated earlier in the prosecution, the claims are not claiming to identify new germline mutations per se, although the claimed methods could certainly be used in such a discovery. Once the methods of this invention are taught to one of skill in the art, that one of skill in the art would use the methods of this invention for any germline mutation selected from the group consisting of truncation-causing mutations, mutations that cause allelic loss, and mutations that cause the expression of proteins with non-wild-type epitopes, wherein said germline mutation is suspected of being associated with a disease or disease susceptibility trait. It would only require routine experimentation by using the claimed methods to determine whether the germline mutation is associated with a disease or disease susceptibility trait in view of conventional knowledge in the art.

Applicant respectfully points out that a "specification is directed to those skilled in the art and need not teach or point out in detail that which is well-known in the art." [In re Myers, 161 USPQ 668, 671 (CCPA 1969); see also, G.E. Col. v. Brenner, 159 USPQ 335 (CAFC 1968).] As the Federal Circuit stated in Spectra-Physics, Inc. v. Coherent, Inc., 3 USPQ2d 1737, 1743 (Fed. Cir. 1987): "A patent need not teach, and preferably omits, what is well known in the art."[1] [Emphasis added.]

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1. See also, Rengo Co. Ltd. v. Molins Mach. Co., 211 USPQ 303, 319 (3d Cir. 1980) wherein the Third Circuit stated, referring to two CCPA opinions [In re Wiggins, 179 USPQ 421, 424-425 (CCPA 1973) and In re Bode, 193 USPQ 12 (CCPA 1977)]:

Applicant respectfully concludes that the instant application reasonably conveys to ones of skill in the art that the Applicant at the time of filing the application had possession of the claimed invention, and that the instant application meets the written description requirement of 35 USC 112, first paragraph. Applicant respectfully requests that the Examiner reconsider and withdraw this rejection in view of the above amendments, remarks and cited case law.

35 USC 103(a) Rejections

The Office Action cites five references (Vogelstein, Markowitz, Liskay, Tavtigian, and Albertsen) in combination with Nozawa, in five 103(a) rejections of the claims. To respond, Applicant first respectfully submits the points on which the methods of the instant application differ from the methods of all of the references cited under 35 U.S.C.103(a) in the Office Action.

First, the claimed methods of this invention can be used to detect many different germline diseases, whereas those of the references cited can be used to detect at most only one germline disease.

Second, none of the references cited teach how to detect a germline mutation in only one allele of a subject gene, using only a single normal biological sample from an apparently unaffected organism, by detecting an about 50% reduction of wild-type protein levels. In the cited references, the loss of a single allele is only detected by nucleic acid based assays. That point is critical, because early intervention

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> It is axiomatic that no description, however detailed, is "complete" in a rigorous sense. Every description will rely to some extent on the reader's knowledge of the terms, concepts, and depictions it embodies. Thus, an understanding of any description will involve some measure of inference. . . . [S]kill in the art can be relied upon to supplement that which is disclosed as well as to interpret what is written.

in a disease or disease susceptibility process is only possible, if detected when only one allele of the subject gene is mutated.

Third, none of the references cited (including Nozawa) teach how to overcome the confounding variables associated with immunoassay testing, such as gel loading, etc. The instant invention addresses those confounding variables by comparing the concentrations of subject wild-type cellular proteins within the same sample, and calculating a ratio of the wild-type proteins expressed by each of at least two genes, preferably, two subject genes that are each candidates for having a germline mutation associated with a such a disease or such a disease susceptibility trait.

Vogelstein

Claims 24-28, 32-35, 37, 39, 43, 55-57and 59-61 stand "rejected under 35 U.S.C. 103(a) as being unpatentable over Vogelstein (U.S. 5,650,281; issued July 22, 1997; effective filing date Jan. 4, 1990) in view of Nozawa (U.S. Patent 5, 328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action, page 6, section 7]. The Office Action further states: "Vogelstein teaches methods for detecting germline mutations in a DCC gene comprising detecting loss of wild-type DCC gene by the detection of loss of expression products of the DCC gene, where the expression product may be a protein molecule, detected by Western blotting. . . ." [Office Action, page 6, section 7.] Applicant respectfully distinguishes the instantly claimed quantitative immunoassay methods from the invention claimed in Vogelstein.

First, Applicant respectfully points out that Vogelstein is directed to the DCC gene, which apparently is not subject to germline mutations. See, for example, Allen 1995, "Molecular biology of colon polyps and colon cancer," which describes mutations in the DCC gene as somatic ones which follow germline mutations in the APC gene, in the LOH pathway of colon carcinogenesis [Semin. Surg. Oncol., 11(6):399-405 (1995)]. Any mutation in the DCC gene would be somatic, and one would not easily find a heterozygotic somatic mutation in the DCC gene in normal biological samples by any methods, let alone protein-based methods. The DCC gene would not be a subject gene in the methods of this present invention, which is directed only to methods of detecting germline mutations.

The instantly claimed methods detect diseases or disease susceptibility traits that are associated with germline mutations in only one allele of a subject gene using only a single normal biological sample from an organism. The word "germline" appears once in Vogelstein at column 9, line 15 in the following quoted paragraph:

> Predisposition to cancers can be ascertained by testing normal tissues of humans for mutations of DCC gene. For example, a person who has inherited a germline DCC mutation would be prone to develop cancers. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells or amniotic fluid for mutations of the DCC gene. Loss of a wild-type DCC allele, whether for example by point mutation or by deletion, can be detected by any of the means discussed above.

[Vogelstein, column 9, lines 15-26; emphasis added.]

As the above-quoted paragraph from Vogelstein states, "a germline DCC mutation . . . can be determined by testing DNA from any tissue. . . . [B]lood can be drawn and DNA extracted from the cells of the blood. . . . Loss of a wild-type DCC allele . . . can be detected by any of the means discussed above. " [*Id*; emphasis added.] It is clear from the paragraph quoted from Vogelstein that the detection of "a germline DCC mutation" is by testing DNA, not by an immunoassay method that determines if "there is only about 50% of the normal level of a wild-type protein" present in a normal biological sample, signifying a germline mutation in one allele of a subject gene as claimed in the instant application.

The above-quoted paragraph from Vogelstein ends with the statement: "Loss of a wild-type DCC allele, whether for example, by point mutation or by deletion, can be detected by any of the means discussed above." [Vogelstein, column 9, lines 23-26; emphasis added.] The "means discussed above" in Vogelstein for detecting the "[l]oss of a wild-type DCC allele" [*id.*] **only concern the use of nucleic acid based assays.** Vogelstein at column 5, lines 19-37 discusses the

> [d]etection of point mutations . . . by molecularly cloning of the allele (or alleles) present in tumor tissue and sequencing that allele(s). . . . Alternatively, the polymerase chain reaction can be used. . . . Insertions and deletions of genes

> can also be detected by these techniques. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score loss of an allele or an insertion in a polymorphic fragment. Other techniques for detecting insertions and deletions as are known in the art can be used.

[Emphasis added.]

In the very next paragraph after the above quote from Vogelstein, the "[l]oss of wild-type genes" is described as being able to "be detected on the basis of the loss of a wild-type expression product of the gene." [Vogelstein, column 5, lines 38-39; emphasis added.] When both alleles are deleted, rather than one allele, the gene is lost, and Vogelstein indicates that the loss of a wild-type gene can be detected by detecting the loss of a wild-type expression product of the gene.

There is no suggestion in Vogelstein of detecting an about 50% decrease in the amount of a wild-type protein expressed by a subject gene as signifying a germline mutation in one allele of that gene. In contrast, Vogelstein only refers to the absence of "wild-type DCC protein" as indicating loss of the wild-type gene. Specifically, Vogelstein states:

> Loss of wild-type DCC genes can also be detected by screening for loss of wild-type DCC protein. For example, monoclonal antibodies immunoreactive with DCC can be used to screen a tissue. Lack of antigen would indicate a DCC mutation. Antibodies specific for mutant alleles could also be used to detect mutant DCC gene product. Such immunological assays could be done in any convenient format known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered DCC protein can be used to detect loss of wild-type DCC genes. Finding a mutant DCC gene product indicates loss of wild-type DCC gene.

[Vogelstein, column 6, lines 46-57; emphasis added.] Loss of wild-type DCC gene can be detected by screening "for loss of wild-type DCC protein" or by detecting the presence of "mutant" or "altered DCC protein." It is the absence of the wild-type DCC protein that signifies in Vogelstein the loss of the wild-type DCC gene, i.e., the absence of both wild-type DCC alleles.

Applicant respectfully submits that it is not obvious how one of skill in the art would employ an immunoassay to detect a germline mutation in one allele of a subject gene, as claimed in the instant application. If there were no wild-type protein present in a sample, one would reasonably assume that neither allele is expressing wild-type protein. However, if a reduced amount of wild-type protein is present in a sample, to what does one compare that amount in view of the confounding variables associated with immunoassay testing, such as gel loading problems, different reagents, and varying cell cycle stages? The instant invention addresses and overcomes those confounding variables, by comparing the concentrations of subject wild-type cellular proteins within the same sample, and calculating a ratio of the wild-type proteins expressed by each of two subject genes, preferably, two genes that have a related function and that are each candidates for having a germline mutation associated with a disease or disease susceptibility trait.

The immunoassay methods of the instant invention are

> based on the theory that normal individuals . . . have 100% expression of . . . [the] subject wild-type gene product and also 100% expression of a wild-type reference gene product. In contrast, an individual with a mutation in an allele of the same gene will in theory have only 50% expression of the subject wild-type gene product, while maintaining 100% expression of the reference wild-type gene product.
>
> . . . The immunoassay methods of this invention are also in this aspect premised on the assumption that germline mutations in two different genes of one individual are very rare.

[Application, page 5, line 24 to page 6, line 2.]

The latter premise of the immediately above quote is significant, especially in preferred embodiments of the instant invention, represented by detecting susceptibility to hereditary non-polyposis colon cancer (HNPCC). A number of mismatch repair (MMR) genes, particularly, MLH1, MSH2, PMS1, PMS2 and MSH6, are candidates for mutations associated with HNPCC or susceptibility to HNPCC. The internal standard methods of this invention using two MMR genes, such as, MLH1 and MSH2, for detecting a germline mutation in one or the other, would not work unless the

premise were correct that "germline mutations in two different genes of one individual are very rare." [*Id.*]

Applicant respectfully disagrees with the Examiner's statement at pages 6 and 7 of the Office Action that "it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have made the claimed methods, because Vogelstein supplies the teaching that DCC gene product is lost or diminished in cancer, especially colorectal cancer and because methods for specifically quantitating antigens of interest . . . [are] known in the art." Applicant respectfully reiterates that Vogelstein only refers to the "loss of wild-type DCC protein" as associated with "the loss of the wild-type gene," i.e., a homozygous condition of losing both wild-type alleles. As detailed above, the loss of a single allele in Vogelstein is only detected by nucleic acid based assays.

Vogelstein states at column 6, line 58 to column 7, line 3:

> <u>Mutant DCC genes or gene products can also be detected in other human body samples, such as serum, stool, urine and sputum</u>. The same techniques discussed above for detection of mutant DCC genes or gene products in tissues can be applied to other body samples. <u>Cancer cells are sloughed off from tumors and appear in such body samples</u>. In addition, the DCC gene product itself may be secreted into the extracellular space and found in these body samples even in the absence of cancer cells. By screening such body samples, a simple early diagnosis can be achieved for many types of cancers. In addition, the progress of chemotherapy or radiotherapy can be monitored more easily by <u>testing such body samples for mutant DCC genes or gene products</u>.

[Emphasis added.]

Whereas the instantly claimed methods only concern testing for <u>germline</u> mutations by assaying for <u>full-length wild-type protein of a subject gene in normal biological samples</u>, Vogelstein begins: "[T]he invention relates to detection of the loss and/or alteration of wild-type DCC genes <u>in tumor tissues</u>." [Vogelstein, column 1, lines 17-19; emphasis added.] As indicated in the above quote from Vogelstein concerning "[m]utant DCC genes or gene products," it is "mutant DCC genes or gene products" or "cancer cells" that "appear in such body samples" as "serum, stool, urine and sputum."

Vogelstein at column 5, lines 9-11 states: "In order to detect the loss of the wild-type DCC gene in a tissue, it is helpful to isolate the tissue free from surrounding normal tissues." It is clear from that statement in Vogelstein that the tissue "free from surrounding normal tissues" is tumor tissue.

In general then Vogelstein is not concerned with testing "normal biological samples" as in the instant invention. Only in Vogelstein at column 3, lines 1-7 and at column 9, line 15-26, is the testing of "normal" samples suggested, and as detailed above, loss of a DCC wild-type gene is only indicated by absence of the wild-type DCC gene or expression products, whereas loss of an allele of a DCC gene is only detected by nucleic acid based assays. Further, the only time "germline mutation" is mentioned in Vogelstein is in association with "testing DNA from any tissue of the person's body." [Vogelstein, column 9, lines 15-20.]

Applicant further respectfully submits that ones of skill in the art would not be using the complex nucleic acid based assays now used to detect germline mutations in one allele of a subject gene, if it were obvious how to use an immunoassay method to detect such a germline mutation. The Background of the instant application points out that "unknown mutations are very difficult to detect by molecular genetic tests." [Application, page 3, lines 28-29.] Further, "the results of molecular genetic assays often cannot be obtained quickly, for example, prior to surgery. Although molecular genetic tests can be performed on cancer tissue samples, the question whether the mutation in the cancer cells was acquired or germline would not be answered." [Application, page 4, lines 26-29.] The quantitative immunoassay methods of this invention, unlike nucleic acid based assays,

> can be done in any pathology laboratory and can be developed to be cost-effective to screen large numbers of individuals in a short amount of time. The assays can be performed quickly, and the results are immediately obtainable. Once the change in the product of a particular gene is identified by the immunoassay methods of this invention, molecular genetic tests can then be employed to determine the precise location of the mutation.

[Application, page 5, lines 10-15.]

In summary, the instantly claimed methods can be distinguished from the methods of Vogelstein in regard to the following points:

(1) rather than detecting the loss of a wild-type DCC gene by detecting the absence of wild-type DCC protein, the instantly claimed methods detect a disease or a disease susceptibility trait associated with a germline mutation in one allele of a subject gene by determining that there is an abnormally low level (about 50% of normal) of wild-type protein in a single normal biological sample from an organism;

(2) a single normal biological sample is assayed in the instantly claimed immunoassay methods, whereas the Vogelstein methods primarily focus upon colonic tumor samples matched to normal colonic mucosa samples;

(3) when Vogelstein briefly focuses upon "normal" tissues, only the absence of DCC wild-type protein, detected by immunoassay, indicates the absence of the wild-type DCC gene;

(4) Vogelstein does not suggest the use of one sample from the same organism, quantifying cellular wild-type protein concentrations, and the use of a ratio of the wild-type protein amounts to address the confounding immunoassay variables that are addressed by the instantly claimed methods; and

(5) the instantly claimed invention concerns detecting a germline mutation in a subject gene or in one of two or more subject genes, wherein the germline mutation is associated with a disease or a disease susceptibility trait, whereas Vogelstein only concerns one gene - the DCC gene, and there have been no published reports that DCC mutations are inherited. DCC is not then within the scope of claims of the instant application in that only germline mutations are detected by the methods of this invention. Moreover, it is unclear that the DCC gene has an etiologic role in sporadic colorectal cancer. [Fazeli et al., Nature, 386: 796-804 (April 1997).]

Nozawa

The Office Action at page 6, section 7 states:

> Nozawa teaches a method for the quantification of an endometrial cancer associated antigen by relating the measured amount of the endometrial associated antigen to the measured amount of a second protein. . . .

> Therefore, it would have been prima facie obvious . . . to have made the claimed methods. . . ."

Applicant respectfully counters that all the 103(a) rejections based on Nozawa are not on point in that Nozawa is not analogous art. Nozawa describes the quantification of a cancer antigen isolated from cancer cells, not normal antigen isolated from normal cells. The problems with quantitating antigens for the detection of disease, that Nozawa addresses in the paragraphs to which the Office Action refers, relate to false positives resulting from subjective interpretation of immunohistochemical staining of cells, nonspecific binding of the detecting anti-cancer antigen antibody in normal cells (giving rise to false positives), and the difficulty in collecting samples of a constant volume (and therefore, of a constant cell number) for the cells in question, endometrial cells or tissue from the uterine cavity [Nozawa et al., col. 2, lines 44-51]. None of those problems relates to the present invention.

According to the methods of the present invention, all normal tissues express the antigen in question, that is, the reverse situation from Nozawa. For the preferred type of cells used in the present invention, peripheral blood lymphocytes, a constant volume of cell sample is easily obtainable. The ratio of Nozawa is used to determine the "signal-to-noise" ratio of the specific antigen-antibody staining relative to background staining, in order to eliminate findings of false positives resulting from background staining (due to nonspecificity of antibody, interfering components in the specimen, etc.). Therefore, the cutoff value of Nozawa et al. refers to ratios above a certain "apparent cancer antigen"-to-housekeeping antigen ratio, representing background noise. Claim 5 of Nozawa reads: "wherein a positive result is indicated by a ratio larger than a predetermined value."

The present invention, however, does not use the ratio as a lower limit cutoff, but a defining value of about 50%, indicating that the ratios of Nozawa et al. and the present invention refer to two different phenomena. In a normal cell sample, in the ratio of **y/x** of Nozawa, the signal intensity **y** would represent nonspecific anti-cancer antigen antibody binding to normal cell components (**Ab1**), relative to signal intensity **x**

representing primarily specific antibody binding to a housekeeping gene product (**Ab2**). Therefore, in a normal cell, the Nozawa ratio might be designated:

$$\frac{\text{Nonspecific } \mathbf{Ab1} \text{ binding}}{\text{Specific } \mathbf{Ab2} \text{ binding} + \text{nonspecific } \mathbf{Ab2} \text{ binding}}$$

According to the present invention, in a normal cell sample, both components of the ratio represent primarily specific antibody binding:

$$\frac{\text{Specific } \mathbf{Ab1} \text{ binding} + \text{nonspecific } \mathbf{Ab1} \text{ binding}}{\text{Specific } \mathbf{Ab2} \text{ binding} + \text{nonspecific } \mathbf{Ab2} \text{ binding}}$$

Here, the ratio is used to determine the normal levels of wild-type protein expression of the subject gene in normal cells.

To summarize, the use of a ratio of antigens in Nozawa et al. is different from that of the present invention, and is nonanalogous art. Applicant respectfully submits that there is nothing in Nozawa that adds to the disclosure of Vogelstein to render the present invention obvious.

Applicant respectfully concludes that neither Vogelstein alone or in view of Nozawa renders the instantly claimed invention obvious, but instead as explained above is evidence of the nonobviousness of the instant invention. Applicant respectfully requests that the Examiner reconsider the instant rejection in view of the above remarks, and withdraw the instant 103(a) rejection.

Markowitz

Claims 24-28, 32-35, 37-40, 43, 44, 55-57and 59-61 stand "rejected under 35 U.S.C. 103(a) as being unpatentable over Markowitz (U.S. 5,866,323; issued Feb. 2, 1999; effective filing date May 22, 1995) in view of Nozawa (U.S. Patent 5, 328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action, page 7, section 8]. The Office Action further states at pages 7 to 8:

> Markowitz teaches methods for detection of TGF-beta RII gene mutations by the detection of protein loss. . . .
>
> Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have made the claimed methods. . . .

Applicant respectfully traverses that rejection by first distinguishing the instant application and the Markowitz reference.

The Markowitz case is similar to that of Vogelstein, discussed above, in that the protein assays to screen for a disease or a disease susceptibility trait are based only on finding a <u>total loss</u> of wild-type RII protein, and Markowitz does not teach the need for quantification of wild-type protein. [Markowitz, col. 2, lines 25-65.] Markowitz at col. 1, lines 61-63 state: "It is an object of this invention to provide a method for diagnosis or prognosis of cancer by **<u>detection of the absence</u>** of functional TGFβ receptor in cells of a patient." [Emphasis added.]

Markowitz teaches the "[u]se of RII inactivation assay for diagnosis and prognosis" [Markowitz, col. 17, line 12], RII being "a colon cancer suppressor gene which is inactivated in 25% of colon cancers." [Markowitz, col. 7, lines 13-15.] The passage in Markowitz, to which the Examiner refers, is entitled "Absence of Wild-Type RII Protein," and in contrast to the subject invention, Markowitz only describes methods to determine either the <u>presence</u> or <u>absence</u> of wild-type RII protein, or the <u>presence</u> or <u>absence</u> of mutant RII protein.

> . . . <u>[F]unctional RII receptor must be present</u> in the cell membrane and at the cell surface to mediate TGF-β effects, and therefore inactivation of the TGF-β growth suppression can be monitored by any method for <u>detecting the absence of functional RII</u> on the cell surface. The <u>presence of RII</u> can be measured by immunoassay, using antibodies specific for RII. . . . Lack of antibody binding would indicate the absence of functional RII molecules. <u>Inactive RII receptors may also be detected</u> by using anti-RII antibodies to detect either aberrant cellular location (e.g., on immunohistochemistry) or by virtue of aberrant molecular size (e.g., on Western Blot assays). Alternatively, antibodies specific for the inactive mutant forms of RII described below may be used to <u>detect the presence of inactive RII</u> directly.

[Markowitz, col. 12, lines 44-61; emphasis added.]

A patient would need to be <u>homozygous</u> for the mutant RII protein in order to be identified by the methods according to Markowitz. Furthermore, although Claims 10-13 and 20 of Markowitz describe "a screening method to aid in diagnosing genetic susceptibility to cancer in a patient," the methods taught in the claims only refer to

detecting the "presence or absence of mutant forms of RII" as being indicative of genetic susceptibility to cancer.

Whereas Nozawa teaches the use of a ratio of antigens in their system, the use of a ratio of antigens in Nozawa is different from that of the present invention, and is nonanalogous art as explained in detail above. Applicant respectfully relies upon the explanation above concerning how the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention.

Applicant respectfully concludes that neither Markowitz alone or in view of Nozawa renders the instantly claimed invention obvious, but instead as explained above is evidence of the nonobviousness of the instant invention. Applicant respectfully requests that the Examiner reconsider the instant rejection in view of the above remarks, and withdraw the second 103(a) rejection.

Liskay

Claims 24-28, 32-35, 37-41, 43, 44, 55-57and 59-61 stand "rejected under 35 U.S.C. 103(a) as being unpatentable over Liskay (U.S. 6,165,713; issued Dec. 26, 2000; effective filing date Dec. 9, 1994) in view of Nozawa (U.S. Patent 5, 328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action, page 8, section 9]. At page 9, the Office Action states:

> Liskay teaches methods of diagnosing a DNA mismatch repair abnormality in a human subject, comprising detecting whether there is an abnormal deficiency of an hMLH1 or hPMS1 protein in a sample. . . .
>
> Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have made the claimed methods. . . .

Applicant respectfully traverses that rejection by first distinguishing the instant application and the Liskay reference.

The Liskay case has many similarities to the Vogelstein case discussed in detail above. Liskay describes the use of antibodies to mutL homolog proteins to detect MLH1 or PMS1 mutations. Like Vogelstein, Liskay teaches the use only of DNA-based tests to screen for disease susceptibility caused by mutations in one allele, as

evidenced by the section entitled "DNA-Based Testing," which states at col. 28, line 61 to col. 29, line 28:

> Tests will be developed based on the determination of the most common mutations for the major genes underlying HPNCC....Labeled probes are then used to analyze the PCR sample for the presence of the disease-causing allele....All tests will depend ultimately on accurate and complete structural information relating to hMLH1, hMSH2, hPMS1 and other DNA mismatch repair genes implicated in HPNCC.

Further, Liskay points out at col. 28, lines 54-57 that "<u>[i]nitial testing</u>, including identifying likely HNPCC families by standard diagnosis and family history study, <u>will likely be done in</u> local and smaller <u>DNA diagnosis laboratories</u>." [Emphasis added.] Why would one perform expensive and time-consuming DNA-based genetic screening, if a rapid and inexpensive protein assay were available?

As pointed out previously in detail regarding Vogelstein, Liskay focuses on tumor samples, and only gives one example of a protein-based assay, that is, immunostaining frozen <u>tumor</u> specimens for "altered or reduced signal," in order to diagnose a possible hereditary basis of the cancer. In the section entitled "Protein Detection-Based Screening," Liskay states:

> Tests based on the <u>functionality</u> of the protein product, per se, may also be used. The protein-examining tests will most likely utilize antibody reagents specific to either the hMLH1, hPMS1 and hMSH2 proteins or other related "cancer" gene products as they are identified.
>
> For example, a frozen <u>tumor</u> specimen can be cross-sectioned and prepared for antibody staining using indirect fluorescence techniques. Certain gene mutations are expected to <u>alter or destabilize the protein structure</u> sufficiently such as to give an <u>altered or reduced</u> signal after antibody staining. It is likely that such tests will be performed in cases where gene involvement in a family's cancer has yet to be established. We are in the process of developing diagnostic monoclonal antibodies against the human MLH1 and PMS1 proteins. We are overexpressing MLH1 and PMS1 human proteins in bacteria. We will purify the proteins, inject them into mice and derive protein specific monoclonal antibodies which can be used for diagnostic and research purposes.

[Liskay, col. 29, lines 29-49; emphasis added.]

Here, Liskay suggests the use of specific antibodies to hMLH1, hPMS1 and hMSH2 proteins, but the "tests are based on the functionality of the protein product," that is, the tests are designed to detect deficiencies in protein function because of mutations which cause them to have an altered structure. Nothing in the section entitled "Protein Detection-Based Screening" refers to quantifying amounts of wild-type protein. Whereas Liskay describes the use of immunological methods which screen for "altered or reduced signal after antibody staining" [Liskay, col. 29, lines 38-40], there is no description in Liskay of what constitutes "altered or reduced signal" of the relevant gene product.

Further, whereas Liskay claims the use of antibodies to mutL homolog proteins to detect MLH1 or PMS1 mutations by "abnormal deficiency" of gene product in a sample from a human subject (see claims 31-40 and claims 50-53), and thereby diagnose a DNA mismatch repair abnormality, Liskay does not define "abnormal deficiency," nor does Liskay indicate that such a protein-based method can be used to screen for genetic predisposition to cancer. The phrase "abnormal defiency" appears nowhere in the Liskay et al. specification, nor does the word "deficiency" alone.

The only discussion of "Protein-Detection-Based Screening" in Liskay is that as quoted above at col. 29 of Liskay. Applicant respectfully submits that the "abnormal deficiency" to which claims 31-40 and claims 50-53 of Liskay refer in the context of the "Protein Detection Based Screening" concerns an abnormality in "the functionality of the protein product" [Liskay, col. 29, line 30]. Those assays in the context of the Liskay specification would apparently be detecting "non-wild-type" protein, inapposite to the instantly claimed methods which detect "wild-type" protein.

Liskay at col. 7, lines 34-44 defines "non-wild-type" as follows:

> . . . --The term "non-wild-type" when applied to nucleic acids and proteins of the present invention, means a version of a nucleic acid or protein that functions in a manner distinguishable from a naturally-occurring, normal version of that nucleic acid or protein. Non-wild-type alleles of a nucleic acid of the invention may differ structurally from wild-type alleles of the same nucleic acid in any of a variety of ways

> including, but not limited to, differences in the amino acid sequence of an encoded polypeptide and/or differences in expression levels of an encoded nucleotide transcript of polypeptide product.

[Emphasis added.]

Whereas Nozawa teaches the use of a ratio of antigens in their system, the use of a ratio of antigens in Nozawa et al. is different from that of the present invention, and is nonanalogous art. Applicant respectfully relies upon the explanation above concerning how the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention.

Applicant respectfully concludes that neither Liskay alone or in view of Nozawa renders the instantly claimed invention obvious, but instead as explained above is evidence of the nonobviousness of the instant invention. Applicant respectfully requests that the Examiner reconsider the instant rejection in view of the above remarks and withdraw the instant 103(a) rejection.

<u>Tavtigian</u>

Claims 24-28, 32-35, 37-40, 43, 44, 55-57and 59-61 stand "rejected under 35 U.S.C. 103(a) as being unpatentable over Tavtigian (U.S. 6,124,104; issued Sep. 26, 2000; effective filing date Apr. 29, 1996) in view of Nozawa (U.S. Patent 5, 328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action, page 10, section 10]. At page 10, the Office Action states: "Tavtigian teaches methods for detection of BRCA2 gene mutations by the detection of protein loss. . . . ," and indicates that Tavtigian in view of Nozawa render the claimed methods prima facie obvious "because Tavtigian supplies the teaching that the BRCA2 gene product is lost or diminished in cancer. . . ." [Office Action, pages 10-11.] Applicant respectfully traverses that rejection by first distinguishing the instant application and the Tavtigian reference.

The instantly claimed immunoassays identify a disease or disease susceptibility trait associated with a germline mutation by detecting <u>wild-type protein</u> expressed by a subject gene at a level of about 50% of the normal level <u>in a normal sample</u>. The assays of the instant invention do not detect <u>mutant</u> proteins expressed by a subject gene, and use the amount of wild-type protein expressed by a subject gene in

ratio either to the amount of wild-type protein expressed by a second subject gene, or to the amount of wild-type protein expressed by a reference gene (that is, wherein the reference gene is not known to be associated with a disease or a disease susceptibility trait). In contrast, Tavtigian, in one aspect, teaches detecting BRCA2 germline mutations by protein assays by identifying mutant BRCA2 proteins in a tissue sample.

For example, Claim 1 of Tavtigian describes

> [a] method for diagnosing a predisposition for breast cancer in a human subject by detecting a germline alteration in the BRCA2 gene in said subject comprising comparing the . . . level of expression of the BRCA2 polypeptide in a tissue sample from said subject with the . . . level of expression of the wild-type BRCA2 polypeptide, wherein an alteration in the . . . level of expression of the BRCA2 polypeptide of said subject indicates a predisposition to said cancer.

Applicant respectfully submits that the interpretation of the first "BRCA2 polypeptide" of Claim 1 must be mutant BRCA2 polypeptide produced by the germline alteration, since it is not qualified by the term "wild-type," as is the second "BRCA2 polypeptide" of Claim 1, and also in view of other evidence in the specification.

Further evidence that "the BRCA2 polypeptide" of Claim 1 of Tavtigian refers to mutant BRCA2 polypeptide is provided in Claim 2 of Tavtigian, which reads:

> 2. The method of claim 1, wherein the detection of a germline alteration comprises (a) sequencing the BRCA2 polypeptide from said sample, (b) screening for the presence of the BRCA2 polypeptide in said sample, (c) screening for a specific alteration in the BRCA2 polypeptide in said sample, or (d) determining the level of expression of the BRCA2 polypeptide in said sample.

If the detection of a germline alteration comprises "screening for the presence of the BRCA2 polypeptide," then "the BRCA2 polypeptide" of Claim 2 must be a mutant BRCA2 polypeptide, as the presence of a wild-type BRCA2 polypeptide would not be indicative of a germline alteration, and the presence of a mutant BRCA2 polypeptide would be indicative of a germline alteration. Also, for a distinction to be made between BRCA2 polypeptide and wild-type BRCA2 polypeptide, the BRCA2 polypeptide must be interpreted as the mutant form.

Still further evidence that "the BRCA2 polypeptide" of Claims 1 and 2 (for which the level of expression is indicative of a germline alteration) refers to mutant BRCA2 polypeptide is provided in Example 7 of Tavtigian [col. 50, lines 1-29], entitled "Generation of Polyclonal Antibody Against BRCA2" which reads in part:

> This procedure is repeated to generate antibodies against the mutant forms of the BRCA2 gene. These antibodies, in conjunction with antibodies to wild type BRCA2, are used to detect the presence and the relative level of the mutant forms in various tissues and biological fluids.

[Tavtigian, col. 50, lines 25-29; emphasis added]. Even though the above-cited example refers to the use of antibodies to wild type BRCA2 protein, it only teaches their usefulness in determining the relative level of the mutant forms in "various tissues and biological fluids."

In the first passage cited by the Examiner relating to protein-based assays [Tavtigian, col. 14, lines 52-67], Tavtigian teaches:

> Alteration of wild-type BRCA2 genes can also be detected by **screening for alteration of wild-type BRCA2 protein**. For example, monoclonal antibodies immunoreactive with BRCA2 can be used to screen a tissue. **Lack of cognate antigen would indicate a BRCA2 mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant BRCA2 gene product.** Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. **Any means for detecting an altered BRCA2 protein can be used to detect alteration of wild-type BRCA2 genes.** Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect BRCA2 biochemical function. **Finding a mutant BRCA2 gene product indicates alteration of a wild-type BRCA2 gene.**

[Emphasis added.] First, as the highlighted phrases from the above quote from Tavtigian emphasize, it is "altered" or "mutant" BRCA2 gene product that is the target of the Tavtigian immunoassays, not wild-type protein, which is the target of the assays of the instant invention. Second, when it comes to amount of BRCA2 gene product detected, Tavtigian detects **"lack of cognate antigen,"** not about 50% of the normal

amount of wild-type protein, as the assays of the instant invention do when a germline mutation is present.

The phrase "lack of cognate antigen," as used in the above-quoted passage cited by the Examiner is not defined by the specification. However, the usage of the term "lack" throughout Tavtigian confirms the Applicant's interpretation of the phrase "lack of cognate antigen" to mean absence of cognate antigen. The term "lack" is used four other times in Tavtigian; each time, it is used in the sense of "absent." Those four usages of "lack" in Tavtigian are as follows (wherein underlining of "lack" is added).

- "All the progeny of such a mutant cell lack the wild type function of BRCA2 and may develop into tumors." [Tavtigian, col. 3, lines 40-42.]
- "In addition, the method can be performed by detecting the wild-type BRCA2 locus and confirming the lack of predisposition to cancer." [Tavtigian, col. 11, lines 24-26.]
- "Lack of total homology may be due to deletions, insertions, inversions or substitutions." [Tavtigian, col. 13, lines 48-49].
- "Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs." [Tavtigian, col. 23, lines 29-32].

The other sections of Tavtigian cited by the Examiner for the "detection of BRCA2 gene mutation by the detection of protein loss . . . " [Office Action, page 10], that is, Tavtigian at "col. 15, line 61 – col. 16, line 10" and "col. 53, lines 20-30" [id.] similarly are inapposite to the claimed methods. Tavtigian at col. 15, line 61 to col. 16, line 10 states:

> Thus, the presence of an altered (or a mutant) BRCA2 gene which produces a protein having a loss of function, or altered function, directly correlates to an increased risk of cancer. In order to detect a BRCA2 gene mutation, a biological sample is prepared and analyzed for a difference between the sequence of the BRCA2 allele being analyzed and the sequence of the wild-type BRCA2 allele. Mutant BRCA2 alleles can be initially identified by any of the techniques described above. The mutant alleles are then sequenced to identify the specific mutation of the particular mutant allele.

> **Alternatively, mutant BRCA2 alleles can be initially identified by identifying mutant (altered) BRCA2 proteins, using conventional techniques.** The mutant alleles are then sequenced to identify the specific mutation for each allele. The mutations, especially those which lead to an altered function of the BRCA2 protein, are then used for the diagnostic and prognostic methods of the present invention.

[Emphasis added.] Again, Tavtigian as shown in the above quote is only concerned with detecting "mutant (altered) BRCA2 proteins" to identify mutant BRCA2 alleles.

Tavtigian at col. 53, lines 20-23 reads:

> BRCA2 is a tumor suppressor gene. A homozygous deletion of this gene may lead to breast cancer as well as other cancers. A homozygous deletion in a pancreatic xenograft was instrumental in the effort to isolate BRCA2 by positional cloning. Cancer may also result if there is a loss of one BRCA2 allele and a mutation in the remaining allele (loss of heterozygosity or LOH). Mutations in both alleles may also lead to development of cancer. For studies here, an analysis of 150 cell lines derived from different cancers revealed no cases in which there was a homozygous loss of the BRCA2 gene.

Applicant respectfully submits that there is no reference in the above quote to "protein loss." Tavtigian only refers in that quote to a homozygous deletion, or to a loss of one BRCA2 allele and a mutation in the other allele. In either situation, no wild-type protein would be expressed. That situation is inapposite to that of the present invention wherein one allele of a subject gene expresses wild-type protein, and it is such wild-type protein which is detected by the immunoassays of the present invention.

Further, other paragraphs in Tavtigian suggest that any assays based on BRCA2 wild-type protein levels would be performed in <u>tumor</u> tissues. For example, Tavtigian at col. 11, lines 44-48 states: "It is believed that many mutations found in <u>tumor</u> tissues will be those leading to decreased expression of the BRCA2 gene product. However, mutations leading to <u>non-functional gene products</u> would also lead to a cancerous state." [Emphasis added.] Also, Tavtigian indicates that the tissue samples would be <u>tumor</u> tissue samples, stating at col. 12, lines 41-51:

> In order to detect the alteration of the wild-type BRCA2 gene in a tissue, it is helpful to isolate the tissue free from surrounding normal tissues. Means for enriching tissue preparation for tumor cells are known in the art. For example, the tissue may be isolated from paraffin or cryostat sections. Cancer cells may also be separated from normal cells by flow cytometer. These techniques, as well as other techniques for separating tumor cells from normal cells, are well known in the art. If the tumor tissue is highly contaminated with normal cells, detection of mutations is more difficult.

[Emphasis added.] If detection of the alteration of the wild-type BRCA2 gene could be performed by finding diminished wild-type BRCA2 protein levels in **normal cells** alone, as in the present invention, why would it be necessary to separate tumor tissue from normal tissue, as Tavtigian and Vogelstein direct?

Tavtigian does not suggest diagnosing disease or disease susceptibility by measuring reduced levels of wild-type BRCA2 protein in normal cells. Tavtigian does not suggest a cutoff value for diagnosing disease or disease susceptibility, nor does Tavtigian suggest using a ratio, another subject gene or an appropriate reference gene, nor does Tavtigian suggest using an about 50% reduction of wild-type BRCA2 as diagnosing susceptibility to breast cancer.

As the Office Action notes on page 10, Tavtigian does not indicate "how the detection of the protein expression will be quantified." The Office Action depends upon the combination of Tavtigian with Nozawa, but as detailed above, Nozawa is not analogous art.

Whereas Nozawa teaches the use of a ratio of antigens, the use of a ratio of antigens in Nozawa is different from that of the present invention, and is nonanalogous art as explained in detail above. Applicant respectfully relies upon the explanation above concerning how the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention.

Applicant respectfully concludes that neither Tavtigian alone or in view of Nozawa renders the instantly claimed invention obvious, but instead as explained above is evidence of the nonobviousness of the instant invention. Applicant respectfully

requests that the Examiner reconsider the instant rejection in view of the above remarks, and withdraw the fourth 103(a) rejection.

Albertsen

Claims 24-28, 32-35, 37-40, 43, 44, 55-57and 59-61 stand "rejected under 35 U.S.C. 103(a) as being unpatentable over Albertsen (U.S. 6,413,727; issued Jul.2, 2002; effective filing date Aug. 8, 1991) in view of Nozawa (U.S. Patent 5, 328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action, page 11, section 11]. At pages 11 to 12, the Office Action states: "Albertsen teaches methods for detection of APC gene mutations by the detection of protein loss . . . ," and indicates that Albertsen in view of Nozawa renders the claimed methods prima facie obvious "because Albertsen supplies the teaching that the APC gene product is lost or diminished in cancer. . . ." Applicant respectfully traverses that rejection by first distinguishing the instant application and the Albertsen reference.

Applicant respectfully submits that Albertsen provides no insights beyond any of the other references cited that could suggest the methods of the instantly claimed invention. The methods of this invention immunologically quantitate the amount of wild-type protein expressed by two or more subject genes, or by a subject gene and a reference gene, in protein extracts from normal cells to detect germline mutations that cause an about 50% decrease in wild-type protein expressed by a subject gene, and thereby the disease or disease susceptibility trait associated with that germline mutation in said subject gene.

Albertsen is very similar to the Vogelstein, Liskay and Tavtigian references. First, as Vogelstein, Liskay and Tavtigian, Albertsen teaches that assays for germline mutations are meant to be performed in tumor tissues. For example, Albertsen at col. 5, lines 49-58 states: "In order to detect the alteration of the wild-type APC gene in a tissue, it is helpful to isolate the tissue free from surrounding normal tissues. . . . If the tumor tissue is highly contaminated with normal cells, detection of mutations is more difficult." [Emphasis added.]

Second, as Vogelstein, Markowitz, Liskay and Tavtigian, Albertsen does not provide a critical value of wild-type APC protein levels for determining genetic

predisposition to cancer. At column 5, lines 29-41, which is the first passage cited in the Office Action, Albertsen states:

> Germline mutations can be found in any of a body's tissues. If only a single allele is somatically mutated, an early neoplastic state is indicated. However, if both alleles are mutated, then a late neoplastic state is indicated. The finding of APC mutations thus provides both diagnostic and prognostic information. . . . It is believed that many mutations found in <u>tumor</u> tissues will be those leading to decreased expression of the APC gene product.

[Emphasis added.] If, as Albertsen believes, "many mutations found in <u>tumor</u> tissues will be those leading to <u>decreased expression</u> of the APC gene product," how does one use protein-based assays to determine whether an individual has a mutation in one APC allele, or mutations in two APC alleles? Do the tumor tissues with "decreased expression of the APC gene product," to which Albertsen refers above, have a single allele mutated, or are both alleles mutated? And how does one detect APC mutations in <u>normal</u> tissues, based on APC gene product, if mutations in <u>tumor</u> tissues lead to "decreased expression" of the APC gene product?

The second passage from Albertsen cited in the Office Action provides no clearer direction on how to differentiate between homozygous and heterozygous APC germline mutations using protein-based assays:

> Alteration of wild-type APC genes can also be detected by **screening for alteration of wild-type APC protein**. For example, monoclonal antibodies immunoreactive with APC can be used to screen a tissue. **Lack of cognate antigen would indicate an APC mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant APC gene product.**

[Albertsen, col. 7, lines 39-43; emphasis added.] Again, it is "lack of cognate antigen" or "mutant" gene product that is being assayed according to Albertsen, not wild-type protein at an about 50% of normal level in normal cells as in the instantly claimed methods.

Albertsen refers at col. 7, lines 39-40 and in Claims 3 and 4 to detecting "alteration of wild-type APC protein." The only examples of detecting "alteration of wild-

type APC protein" is as quoted above from Albertsen at col. 7, lines 39-43, that is where there is "lack of cognate antigen" or a "mutant" APC gene product.

The only other use of the term "lack" in Albertsen is at col. 6, lines 41-45 which reach:

> Mismatches, according to the present invention are hybridized nucleic acid duplexes which **are not 100% homologous. The lack of total homology** may be due to deletions, insertions, inversions, substitutions or frameshift mutations.

[Emphasis added.] That use of "lack of total homology" indicates "absence" of total homology.

The only guidance provided by Albertsen for the use of an APC protein-based assay as diagnostic/prognostic of cancer is <u>vaguely qualititative, not quantitative</u>. For example, Albertsen at col. 5, lines 39-48 states:

> It is believed that **many mutations found in tumor tissues will be those leading to decreased expression of the APC gene product**. However, **mutations leading to non-functional gene products** would also lead to a cancerous state. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, leading to **loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the APC gene product.**

[Emphasis added.] In contrast, the immunoassays of the instant invention can differentiate between homozygous and heterozygous mutations, and provide a definitive answer that could be critical to making a life-saving early intervention. Further, the assays of this invention are directed to germline mutations that are "selected from the group consisting of truncation-causing mutations, mutations that cause allelic loss, and mutations that cause the expression of proteins with non-wild-type epitopes. . . ." [Instant invention's independent Claims 24 and 73.]

Whereas Nozawa teaches the use of a ratio of antigens, the use of a ratio of antigens in Nozawa is different from that of the present invention, and is nonanalogous art as explained in detail above. Applicant respectfully relies upon the

explanation above concerning how the ratio of Nozawa differs substantially from the ratios of the methods of the instant invention.

Applicant respectfully concludes that neither Albertsen alone or in view of Nozawa renders the instantly claimed invention obvious, but instead as explained above is evidence of the nonobviousness of the instant invention. Applicant respectfully requests that the Examiner reconsider the instant rejection in view of the above remarks and case law, and withdraw the fifth 103(a) rejection.

CONCLUSION

Applicant respectfully concludes that the claims as amended are in condition for allowance, and earnestly requests that the claims be promptly allowed. If for any reason the Examiner feels that a telephone conference could be helpful, the Examiner is invited to telephone the undersigned Attorney for Applicant at (415) 981-2034.

Respectfully submitted,

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